36. The polypeptide according to claim 35, wherein the hemolytic activity is less than 25% compared to wild-type pneumolysin.

37. The polypeptide according to claim 36, wherein the hemolytic activity is less than 1% compared to wild-type pneumolysin.

38. The polypeptide according to claim 35, wherein the region comprising amino acid residues 1 to 257 is substituted at one or more residue(s) selected from the group consisting of positions 61, 148, and 195, and the combination of positions 17, 18, 61, 66 and 101; 41, 172, 195 and 255; 63, 127, 128 and 148; 33, 46, 83, 239 and 257; and 45, 102, 189 and 195.

39. The polypeptide according to claim 38, comprising at least one amino acid substitution at residue positions 61, 148, or 195 or the combination of substitutions at residue positions 33, 46, 83, 239 and 257.

40. The polypeptide according to claim 39, wherein a single amino acid substitution is made and the substituted amino acid is selected from the group consisting of proline or hydroxyproline for position 61; lysine, arginine or histidine for position 148 and leucine, glycine, alanine, isoleucine or valine for position 195.

41. The polypeptide according to claim 39, wherein the substituted amino acids are selected from the group consisting of serine, threonine, asparagine, glutamine, tyrosine or cysteine for positions 33, 46 and 83; lysine, arginine or histidine for position 239 and leucine, glycine, alanine, isoleucine or valine for position 257.

42. The polypeptide according to claim 35, wherein the polypeptide is selected from the group consisting of pNVJ1, pNVJ20, pNVJ22, pNVJ45, pNVJ56, pNV103, pNV207, pNV111, and pNV211.

43. Modified pneumolysin polypeptide pNVJ1.

44. Modified pneumolysin polypeptide pNVJ20.

45. Modified pneumolysin polypeptide pNVJ22.

46. Modified pneumolysin polypeptide pNVJ45.

47. Modified pneumolysin polypeptide pNVJ56.

48. Modified pneumolysin polypeptide pNV103.

49. Modified pneumolysin polypeptide pNV207.

50. Modified pneumolysin polypeptide pNV111.

51. Modified pneumolysin polypeptide pNV211.

52. A pneumolysin polypeptide comprising SEQ ID NO: 3, wherein the polypeptide is modified, refolded and has attenuated hemolytic activity, and wherein the polypeptide has at least one amino acid substitution in the region comprising amino acids 1 to 257, and wherein the polypeptide is obtained by:

(a) mutating a nucleic acid molecule encoding a pneumolysin polypeptide;

(b) expressing the mutated nucleic acid molecule in a host cell;

(c) assaying a polypeptide expressed from the mutated nucleic acid molecule for

hemolytic activity;

(d) selecting a polypeptide having attenuated hemolytic activity;

(e) assaying the selected polypeptide for refoldability; and

(f) selecting a refolded polypeptide.

53. The polypeptide according to claim 52, wherein the polypeptide is obtained by randomly mutating a nucleic acid molecule encoding a pneumolysin polypeptide.

54. The polypeptide according to claim 52, wherein the hemolytic activity is less than 25% compared to wild-type pneumolysin.

55. The polypeptide according to claim 54, wherein the hemolytic activity is less than 1% compared to wild-type pneumolysin.

56. The polypeptide according to claim 52, wherein the region comprising amino acid residues 1 to 257 is substituted at one or more residue(s) selected from the group consisting of positions 61, 148, and 195, or the combination of positions 17, 18, 61, 66 and 101; 41, 172, 195 and 255; 63, 127, 128 and 148; 33, 46, 83, 239 and 257; and 45, 102, 189 and 195.

57. The polypeptide according to claim 56, comprising at least one amino acid substitution at residue positions 61, 148, or 195 or the combination of substitutions at residue positions 33, 46, 83, 239 and 257.

58. The polypeptide according to claim 56, wherein a single amino acid substitution is made and the substituted amino acid is selected from the group consisting of proline or

hydroxyproline for position 61; lysine, arginine or histidine for position 148 and leucine, glycine, alanine, isoleucine or valine for position 195.

59. The polypeptide according to claim 56, wherein the substituted amino acids are selected from the group consisting of serine, threonine, asparagine, glutamine, tyrosine or cysteine for positions 33, 46 and 83; lysine, arginine or histidine for position 239 and leucine, glycine, alanine, isoleucine or valine for position 257.

60. The polypeptide according to claim 52, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

61. The conjugate according to claim 60, wherein the polysaccharide is derived from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B or C; group B streptococcus type Ia, Ib, II, III, V or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.

62. A vaccine comprising the polypeptide according to claim 52 and a pharmaceutically acceptable carrier.

63. The vaccine according to claim 62, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

64. The vaccine according to claim 63, wherein the polysaccharide is a bacterial polysaccharide and is derived from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus type Ia, Ib, II, III, V, or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.